



Short communication

Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection

Isabela da Costa César*, Fernando Henrique Andrade Nogueira, Gérson Antônio Pianetti

Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:

Received 7 February 2008

Received in revised form 9 May 2008

Accepted 20 May 2008

Available online 28 May 2008

Keywords:

Artemether

Lumefantrine

Antimalarials

HPLC-UV

Fixed dose combination tablets

ABSTRACT

This paper describes the development and validation of a HPLC-UV method (210 nm) for the simultaneous quantitation of artemether and lumefantrine in fixed dose combination tablets. The method showed to be linear ($r^2 > 0.99$), precise (R.S.D. $< 2.0\%$), accurate (recovery of 101.07% for artemether and 101.58% for lumefantrine), specific and robust. Four batches of artemether–lumefantrine tablets were assayed by the validated method. The artemether contents in the tablets varied from 98.61% to 103.35%, while lumefantrine contents were 97.92–100.48%.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the poor countries of the world [1]. One of the greatest challenges facing malaria control worldwide is the spread and intensification of parasite resistance to antimalarial drugs. The limited number of such drugs has led to increasing difficulties in the development of antimalarial drug policies and adequate disease management [2].

Artemisinin-based combination therapy (ACT) is increasingly being advocated as promising treatment. ACT is based on the use of two drugs with different modes of action: an artemisinin-derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action [3].

Artemether–lumefantrine is an ACT widely used nowadays and consists of a registered fixed dose combination of artemether (20 mg) and lumefantrine (120 mg) in tablets (Fig. 1). The rationale is that artemether will rapidly reduce parasitemia, resulting in symptomatic relief, and lumefantrine will eliminate the remaining parasites [4]. World Health Organization (WHO) recommends this association as first line therapy for falciparum malaria in endemic areas [5].

The increasing use of artemether–lumefantrine association as an effective treatment for resistant malaria demands the need of analytical methods to simultaneously quantify these drugs in tablets in order to evaluate its quality. Some papers have described the analysis of artemether in plasma, based on HPLC with electrochemical [6–8] or mass spectrometry detection [9]. Few methods are available to assay artemether in pharmaceutical products [10,11]. The quantitative determination of lumefantrine in plasma has been described using HPLC with UV detection [12–15]. However, there is no method reported regarding the simultaneous quantitation of artemether and lumefantrine.

Hence, the aim of this study was to develop and validate a HPLC method, using UV detection, to simultaneously quantify artemether and lumefantrine in fixed dose combination tablets. Due to the low molar absorptivity of artemether in the UV region (210 nm), and the lower concentration of this drug in the tablets compared to lumefantrine, standard addition of artemether was carried out to improve its detection. The validated method was applied to the analysis of tablets containing the artemether–lumefantrine association (20 + 120 mg).

2. Experimental

2.1. Reagents and materials

Artemether and lumefantrine reference standards were purchased from Dafra Pharma (Turnhout, Belgium). Coartem® (Novartis, Basel, Switzerland) tablets were kindly donated by

* Corresponding author. Tel.: +55 31 34096982; fax: +55 31 34096976.
E-mail address: isaccesar@bol.com.br (I.d.C. César).

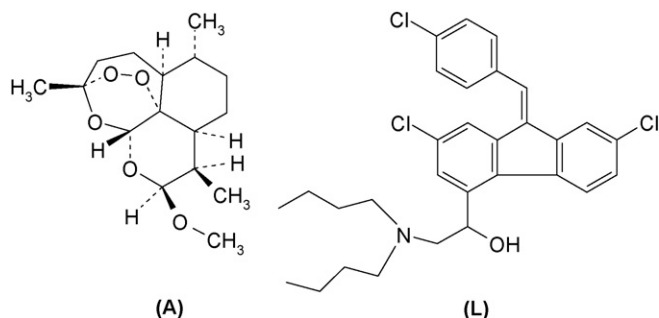


Fig. 1. Chemical structures of artemether (A) and lumefantrine (L).

Brazilian Health Ministry and Co-artesiane® tablets were purchased from Dafra Pharma (Turnhout, Belgium). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile, trifluoroacetic acid and chloroform (HPLC grade) were obtained from Tedia (Fairfield, OH, USA).

2.2. Instrumental and analytical conditions

The HPLC analyses were carried out on an Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, autosampler, diode array detector (DAD) and HP ChemStation software. The columns evaluated were a Zorbax SB-C18 (150 × 4.6 mm i.d.; 5 µm particle size) from Agilent (Santa Clara, CA, USA) and a Symmetry C₁₈ (250 × 4.6 mm i.d.; 5 µm particle size) from Waters (Milford, MA, USA), both maintained at 30 °C. UV detection was performed at 210 nm. UV spectra from 190 to 400 nm were online recorded for peak identification. The injection volume was 20 µl. An isocratic mobile phase containing acetonitrile and 0.05% trifluoroacetic acid (60:40, v/v) was used at a flow rate of 1.0 ml/min. The separation of artemether and lumefantrine was evaluated in different proportions of these solvents and, for each condition, retention factor (*k*) and resolution (*R*) were calculated. In order to determine *k*, *t*₀ was estimated by injecting a 0.01% (w/v) NaNO₃ solution in mobile phase onto the chromatograph. The optimized condition was achieved using the Zorbax SB-C18 column and a mobile phase composed of acetonitrile and 0.05% trifluoroacetic acid (60:40), pH 2.35.

2.3. Preparation of standard solutions

2.3.1. Artemether–lumefantrine standard solution

Approximately 40 mg of artemether and 30 mg of lumefantrine reference standards were accurately weighed and transferred to a 100 ml volumetric flask. Chloroform (2 ml) was added to ensure complete solubilization, followed by the addition of 80 ml of acetonitrile. The volume was filled to the mark with 0.05% trifluoroacetic acid, to obtain a solution at 400 µg/ml of artemether and 300 µg/ml of lumefantrine.

2.3.2. Artemether stock solution

Approximately 175 mg of artemether reference standard was accurately weighed and transferred to a 100 ml volumetric flask. Chloroform (2 ml) was added to ensure complete solubilization and the flask volume was completed with acetonitrile. The final concentration was 1750 µg/ml of artemether.

2.3.3. Artemether work solution

An aliquot of 10 ml of artemether stock solution was transferred to a 50 ml volumetric flask and the volume was adjusted with mobile phase, to obtain a solution at 350 µg/ml of artemether.

2.4. Analysis of fixed dose combination tablets

Three different batches of Coartem® and one batch of Co-artesiane® were analyzed using the validated method. Artemether standard was added to the samples, with the aim of increasing the peak area of artemether in the chromatograms and thereby improving the detection of this compound. Due to the poor solubility of lumefantrine, chloroform was added to ensure the complete solubilization of the samples. For the analysis, six replicates of each batch were assayed. The tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 25 mg of artemether and 150 mg of lumefantrine, was transferred to a 100 ml volumetric flask followed by the addition of 5 ml of chloroform. The solution was sonicated for 3 min and diluted with acetonitrile to volume. An aliquot of 10 ml of this solution was transferred to a 50 ml volumetric flask and 10 ml of the artemether stock solution (described in Section 2.3) was added. The volume was adjusted with mobile phase. An artemether work solution was also prepared by diluting 10 ml of the artemether stock solution to 50 ml with mobile phase. To calculate artemether content in the tablets, the peak area of artemether obtained in the artemether work solution was subtracted from the peak area of artemether obtained in the sample solutions. The difference corresponds to the amount of artemether originally present in the tablet samples.

2.5. Validation

2.5.1. Linearity

Standard solutions containing 750 µg/ml of lumefantrine were prepared, in triplicate. Aliquots of these solutions were diluted in mobile phase to five different concentrations, corresponding to 150, 225, 300, 375 and 450 µg/ml of lumefantrine. For the artemether, solutions were prepared using the standard addition procedure. Stock sample solutions containing 250 µg/ml of artemether were prepared in triplicate. An aliquot of these sample solutions was diluted in mobile phase and 10 ml of artemether stock solution, corresponding to 350 µg/ml of artemether, was added. Therefore, the final concentrations were 375, 387.5, 400, 412.5 and 425 µg/ml of artemether. Calibration curves for concentration versus peak area were plotted for each compound and the obtained data were subjected to regression analysis using the least squares method with a weighting factor of 1/*x*.

2.5.2. Precision

The intra-day precision was evaluated by analyzing six sample solutions (*n* = 6), at the final concentration of analyses (400 µg/ml of artemether and 300 µg/ml of lumefantrine). Similarly, the inter-day precision was evaluated in three consecutive days (*n* = 18). The artemether and lumefantrine concentrations were determined and the relative standard deviations (R.S.D.) were calculated.

2.5.3. Accuracy

Artemether and lumefantrine reference standards were accurately weighed and added to a mixture of the tablet excipients, at three different concentration levels (300, 400 and 500 µg/ml of artemether and 225, 300 and 375 µg/ml of lumefantrine). At each level, samples were prepared in triplicate and the recovery percentage was determined.

2.5.4. Specificity

Spectral purities of artemether and lumefantrine chromatographic peaks were evaluated using the UV spectra recorded by a diode array detector. In addition, a solution containing a mixture

Download English Version:

<https://daneshyari.com/en/article/1222818>

Download Persian Version:

<https://daneshyari.com/article/1222818>

[Daneshyari.com](https://daneshyari.com)